Amendments to the Claims:

The following listing of claims replaces all previous listings filed in this application.

Listing of Claims:

1. (Currently Amended) An isoelectric gateway for use in the alteration of the composition of a sample comprising:

a first ion-permeable barrier <u>capable of restricting passage of molecules of a specified size</u> <u>having a characteristic pore size substantially restricting passage of molecules having a size larger than the characteristic pore size of the barrier;</u>

a second ion-permeable barrier capable of restricting passage of molecules of a specified sizehaving a characteristic pore size substantially restricting passage of molecules having a size larger than the characteristic pore size of the barrier, wherein the first ion-permeable barrier and the second ion-permeable barrier are selected from the group consisting of porous solids, non-ionic membranes isoelectric membranes, non-ionic gels, and isoelectric gels, and wherein the second ion-permeable barrier is positioned at a distance apart from the first ion-permeable barrier so as to define a space therebetween; and

an isoelectric substance having a characteristic pI value and molecular weights such that the isoelectric substance cannot pass through the ion-permeable barriers, the isoelectric substance a characteristic size larger than the pore size of the ion-permeable barriers disposed in the space between the first and second ion-permeable barriers forming an isoelectric gateway, and wherein the ion-permeable barriers substantially restrict movement of the isoelectric substance through the ion-permeable barriers, and wherein the pI value of the isoelectric gateway remains substantially constant during electrophoresis.

56104576-51

- 2. (Cancelled)
- 3. (Cancelled)
- 4. (Cancelled)
- (Cancelled)
- 6. (Currently Amended) The isoelectric gateway according to claim 51 wherein the first and second ion-permeable barriers are non-ionic membranes are unsupported membranes selected from the group consisting of cellulose esters, polysulfones, polyschersulfones, cross-linked polyacrylates and cross-linked erosslinked polyacrylamides.
- 7. (Currently Amended) The isoelectric gateway according to claim 51 wherein the first and second ion-permeable barriers are non-ionic membranes are supported membranes selected from cross-linked polyacrylamides supported on glass fiber, filter paper, polymeric mesh or paper.
- 8. (Currently Amended) The isoelectric gateway according to claim 4 wherein claim 1 wherein the first and second ion-permeable barriers are porous frits selected from the group consisting of glass frits, ceramic frits, and polymeric frits.
- 9. (Cancelled)
- 10. (Original) The isoelectric gateway according to claim 1 wherein the isoelectric substance is selected from the group consisting of substances containing a combination of weak acid and weak base functionalities, substances containing a combination of weak acid and strong base functionalities, and substances containing a combination of strong acid and weak base functionalities.
- 11. (Previously Presented) The isoelectric gateway according to claim 1 wherein the isoelectric substance is selected from the group consisting of polymers with amino and

carboxylic acid groups, polymers with amino and phenolic groups, polymers with amino and phosphonic acid groups, polymers with amino and sulfonic acid groups, polymers with amino, carboxylic acid and phenolic groups, polymers with amino, carboxylic acid and phosphonic acid groups, polymers with amino, phosphonic acid and phenolic groups, polymers with amino, phenolic and sulfonic acid groups, polymers with amino, phenolic, carboxylic acid and sulfonic acid groups, polymers with amino, phenolic, carboxylic acid, phosphonic acid and sulfonic acid groups or polymers with imino and carboxylic acid groups, polymers with imino and phenolic groups, polymers with imino and phosphonic acid groups, polymers with imino and sulfonic acid groups, polymers with imino, phenolic and carboxylic acid groups, polymers with imino, phenolic and phosphonic acid groups, polymers with imino, carboxylic acid and phosphonic acid groups, polymers with imino, phenolic and sulfonic acid groups, polymers with imino, phenolic, carboxylic acid and sulfonic acid groups, polymers with imino, phenolic, carboxylic acid, phosphonic acid and sulfonic acid groups or polymers with quaternary ammonium groups and carboxylic acid groups, polymers with quaternary ammonium groups and phenolic groups, polymers with quaternary ammonium groups and phosphonic acid groups, polymers with quaternary ammonium groups and sulfonic acid groups, polymers with quaternary ammonium groups, phenolic and carboxylic acid groups, polymers with quaternary ammonium groups, phenolic and phosphonic acid groups, polymers with quaternary ammonium groups, carboxylic acid and phosphonic acid groups, polymers with quaternary ammonium groups, phenolic and sulfonic acid groups, polymers with quaternary ammonium groups, phenolic, carboxylic acid and sulfonic acid groups, polymers with quaternary ammonium groups, phenolic, carboxylic acic, phosphonic acid and sulfonic acid groups, and combinations thereof.

- 12. (Original) The isoelectric gateway according to claim 1 wherein the isoelectric substance has a pI value ranging from about 1 to about 13.
- 13. (Currently Amended) The isoelectric gateway according to claim 1 wherein the isoelectric substance is <u>disposed</u> stationary within the space between the ion-permeable

barriers, is <u>disposed</u> flowing through the space between the ion-permeable barriers, or is <u>disposed</u> flowing through the space between the ion-permeable barriers and recirculated therethrough.

- 14. (Previously Presented) The isoelectric gateway according to claim 13 wherein the isoelectric substance remains substantially stationary within the space between the ion-permeable barriers.
- 15. (Currently Amended) A method for altering the composition of a sample comprising:

communicating an isoelectric substance disposed between a first ion-permeable barr er capable of having a characteristic pore size substantially restricting passage of molecules having of a specified size larger than the characteristic pore size of the barrierand a second ion-permeable barrier having a characteristic pore size substantially capable of restricting passage of molecules having of a specified size larger than the characteristic pore size of the barrier to form an isoelectric gateway, wherein the first ion-permeable barrier and the second ion-permeable barrier are selected from the group consisting of porous solids, non-ionic membranes, isoelectric membranes, non-ionic gels, and isoelectric gels, and molecular weight such that the isoelectric substance has a characteristic pl value and molecular weight such that the isoelectric substance cannot pass through a characteristic size larger than the pore size of the ion-permeable barriers, and wherein the ion-permeable barriers substantially restrict movement of the isoelectric substance through the ion-permeable barriers; and

applying a selected electric potential across associated electrodes positioned on opposing sides of the isoelectric gateway wherebyto cause migration of at least one component movesthrough at least one of the ion-permeable barriers, wherein the pI value of the isoelectric gateway remains substantially constant during application of the selected electric potential.

- 16. (Currently Amended) An electrophoresis system for isoelectric focusing comprising:
 - a first electrolyte chamber containing a first electrode;
- a second electrolyte chamber containing a second electrode, wherein the second electrolyte chamber is disposed relative to the first electrolyte chamber so that the electrodes are adapted to generate an electric field in an electric field area upon application of a selected electric potential between the electrodes;
- a first sample chamber disposed between the first and second electrolyte chambers and proximate to the first electrolyte chamber so as to be at least partially disposed in the electric field area;

a second sample chamber disposed between the first sample chamber and the second electrolyte chamber so as to be at least partially disposed in the electric field area;

a first isoelectric gateway separating the first electrolyte chamber and the first sample chamber, wherein the first isoelectric gateway is comprised of a first ionpermeable barrier having a characteristic pore size substantially capable of restricting passage of molecules havingof a specified size larger than the characteristic pore size of the barrierand a second ion-permeable barrier having a characteristic pore size substantiallycapable of restricting passage of molecules having of a specified size larger that the characteristic pore size of the barrierto form an isoelectric gateway, wherein the second ion-permeable barrier is positioned at a distance apart from the first ionpermeable barrier so as to define a space therebetween, and wherein the first ionpenneable barrier and the second ion-permeable barrier are selected from the group consisting of porous solids, non-ionic membranes isoelectric membranes, non-ionic gels and isoelectric gels, an isoelectric substance disposed in the space between the first and second ion-permeable barriers forming the first isoelectric gateway, wherein the isoelectric substance has a characteristic pI value and molecular weight such that the isoclectric substance cannot pass through the ion-permeable barriersa characteristic size larger than the pore size of lacks a covalent bond to the ion permeable barriers, and wherein the ion-permeable barriers substantially restrict movement of the isoelectric substance through the ion-permeable barriers and substantially prevent convective mixing of the contents of the first isoelectric gateway, the first sample chamber and the first electrolyte chamber;

a first selective barrier separating the first and second sample chambers so as to impede convective mixing between contents of the first and second sample chambers;

a second selective barrier separating the second sample chamber from the second electrolyte chamber so as to impede convective mixing between contents of the second sample chamber and second electrolyte chamber;

means adapted for communicating an associated first electrolyte to the first electrolyte chamber;

means adapted for communicating an associated second electrolyte to the second electrolyte chamber, wherein the pH values of associated first and second electrolytes are different;

means adapted for communicating a first fluid to the first sample chamber; and means adapted for communicating a second fluid to the second sample chamber, wherein at least one of the first and second fluids contains at least a sample; and

means adapted for applying a selected electric potential between the electrodes wherein application of the selected electric potential causes migration of at least one component through at least one of the ion-ion-permeable barriers, and wherein the pl value of the first isoelectric gateway remains substantially constant during application of the selected electric potential.

- 17. (Original) The electrophoresis system according to claim 16 wherein a pH gradient is formed between the electrodes.
- 18. (Currently Amended) The electrophoresis system according to claim 16 wherein the first selective barrier is a second isoelectric gateway which separates the first and

second sample chambers, wherein the second isoelectric gateway is comprised of a third ion-permeable barrier having a characteristic pore size substantially capable of restricting passage of molecules having of a specified size larger than the characteristic pore size of the burrier and a fourth ion-permeable barrier having a characteristic pore size substantiallycapable of restricting passage of molecules having of a specified size larger than the characteristic pore size of the barrierto form an isoelectric gateway, wherein the third ion-permeable barrier and the fourth ion-permeable barrier are selected from the group consisting of porous solids, non-ionic membranes, isoelectric membranes, nonionic gels and isoelectric gels, and wherein the fourth ion-permeable barrier is positioned at a distance apart from the third jon-permeable barrier so as to define a space there between, an isoelectric substance disposed in the space between the third and fourth ion-permeable barriers forming the second isoelectric gateway, wherein the isoelectric substance has a characteristic pI value and molecular weight such that the isoelectric substance cannot pass through the third and fourth ion-permeable barriersa characteristic size larger than the pore size of the third and fourth ion permeable barriers, and wherein the third and fourth ion-permeable barriers substantially restrict movement of the isoelectric substance through the third and fourth ion-permeable barriers and substantially prevent convective mixing between the contents of the second isoelectric gateway, the first sample chamber, and the second sample chamber.

19. (Currently Amended) The electrophoresis system according to claim 16 wherein the second selective barrier is a second isoelectric gateway which separates the second sample chamber from the second electrolyte chamber, wherein the second isoelectric

gatev/ay is comprised of a third ion-permeable barrier having a characteristic pore size substantially capable of restricting passage of molecules having of a specified size larger than the characteristic pore size of the barrier and a fourth ion-permeable barrier having a eharueteristic pore size substantially capable of restricting passage of molecules having of a specified size larger than the characteristic pore size of the barrierto form an isoelectric gateway, wherein the third ion-permeable barrier and the fourth ion-permeable barrier are selected from the group consisting of porous solids, non-ionic membranes, isoelectric membranes, non-ionic gels, and isoelectric gels, and wherein the fourth ion-permeable barrier is positioned at a distance apart from the third ion-permeable barrier so as to define a space therebetween, an isoelectric substance distposed in the space between the third and fourth ion-permeable barriers forming the second isoelectric gateway, wherein the isoelectric substance has a characteristic pI value and molecular weight such that the isoelectric substance cannot pass through the third and fourth ion-permeable barriersa characteristic size larger than the pore size of the third and fourth ion-permeable barriers, and wherein the third and fourth ion-permeable barriers substantially restrict movement of the isoelectric substance through the third and fourth ion-permeable barriers and substantially prevent convective mixing between the contents of the second isoelectric gateway, the second sample chamber, and the second electrolyte chamber.

20. (Currently Amended) The electrophoresis system according to claim 16 wherein the first selective barrier is a second isoelectric gateway which separates the first and second sample chambers, wherein the second isoelectric gateway is

comprised of a third ion-permeable barrier-having a characteristic pore size substantially capable of restricting passage of molecules having of a specified size kirger than the characteristic pore size of the barrier and a fourth ion-permeable barrier having a characteristic pore size substantially capable of restricting passage of molecules having of a specified size larger than the characteristic pere size of the barrier to form an isoelectric gateway, wherein the third ion-permeable barrier and the fourth ion-permeable barrier are selected from the group consisting of porous solids, ron-ionic membranes, isoelectric membranes, non-ionic gels, and isoelectric gels, and wherein the fourth ion-permeable barrier is positioned at a distance apart from the third ion-permeable barrier so as to define a space therebetween, an isoelectric substance disposed in the space between the third and fourth ion-permeable barriers forming the second isoelectric gateway, wherein the isoelectric substance has a characteristic pI value and molecular weight such that the isoelectric substance cannot pass through the third and fourth ion-permeable barriersa characteristic size larger han the pore size of the third and fourth ion-permeable barriers, and wherein the third and fourth ion-permeable barriers substantially restrict movement of the isoelectric substance through the third and fourth ion-permeable barriers and substantially prevent convective mixing between the contents of the second isoelectric gateway, the first sample chamber, and the second sample chamber; and

the second selective barrier is a third isoelectric gateway which separates the second sample chamber from the second electrolyte chamber, wherein the third isoelectric gateway is comprised a fifth ion-permeable barrier having a characteristic pere size substantially capable of restricting passage of molecules having of a

specified size larger than the characteristic pore size of the barrer and a sixth ionpermeable barrier having a characteristic pore size substantially capable of restricting passage of molecules having of a specified size larger than the characteristic pore size of the barrierto form an isoelectric gateway, wherein the fifth ion-permeable barrier and the sixth ion-permeable barrier are selected from the group consisting of porous solids, non-ionic membranes, isoelectric membranes, non-ionic gels, and isoelectric gels, and wherein the sixth ion-permeable barrier is positioned at a distance apart from the fifth ion-permeable barrier so as to define a space therebetween, an isoelectric substance disposed in the space between the third and fourth ionpermeable barriers forming the third isoelectric gateway, wherein the isoelectric substance has a characteristic pI value and molecular weight such that the isoelectric substance cannot pass through the fifth and sixth ion-permeable barriersa characteristic size larger than the pore size of the fifth and sixth ion-permeable barriers, and wherein the fifth and sixth ion-permeable barriers substantially restrict movement of the isoelectric substance through the fifth and sixth ion-permeable barriers and substantially prevent convective mixing between the contents of the third isoelectric gateway, the second sample chamber, and the second electrolyte chamber.

21. (Currently Amended) A method for isoelectric focusing comprising:

communicating a first electrolyte to a first electrolyte chamber containing a first electrode;

communicating a second electrolyte to a second electrolyte chamber containing a second electrode, wherein the second electrolyte chamber is disposed relative to the first

56104576-51

electrolyte chamber so that the electrodes are adapted to generate an electric field in an electric field area upon application of a selected electric potential between the electrodes;

communicating a first fluid to a first sample chamber disposed between the first and second electrolyte chambers and proximate to the first electrolyte chamber so as to be at least partially disposed in the electric field area;

communicating a second fluid to a second sample chamber disposed between the first sample chamber and the second electrolyte chamber so as to be at least partially disposed in the electric field area, wherein a first isoelectric gateway separates the first electrolyte chamber and the first sample chamber, wherein the first isoelectric gateway is comprised of a first ion-permeable barrier having a characteristic pore size substantiallycapable of restricting passage of molecules having of a specified size larger than the characteristic pore size of the barrier and a second ion-permeable barrier having a characteristic pore size substantially capable of restricting passage of molecules havingof a specified size larger than the characteristic pore size of the barrierto form an isoel setric gateway, wherein the second ion-permeable barrier is positioned at a distance apart from the first ion-permeable barrier so as to define a space therebetween, and wherein the first ion-permeable barrier and the second ion-permeable barrier are selected from the group consisting of porous solids, non-ionic membranes isoelectric membranes, non-ionic gels and isoelectric gels, an isoelectric substance disposed in the space between the first and second ion-permeable barriers forming the first isoelectric gateway, wherein the isoelectric substance has a characteristic pI value and molecular weight such that the isoelectric substance cannot pass through the first and second ion-permeable barrersa characteristic size larger than the pore size of the first and second ion permeable barriers, and wherein the first and second ion-permeable barriers substantially restrict movement of the isoelectric substance through the first and second ion-permeable barriers and substantially prevent convective mixing between the contents of the first isoelectric gateway, the first sample chamber and the first electrolyte chamber, a first selective barrier separates the first and second sample chambers so as to impede convective mixing between the contents of the first and second sample chambers, and a second selective barr.er separates the second sample chamber and the second electrolyte chamber so as to impede convective mixing between the contents of the second sample chamber and the

second electrolyte chamber, wherein at least one of the first and second fluids contains a samp e; and

applying a selected electric potential to cause migration of at least one selected component through at least one ion-ion-permeable barrier, and wherein the pI value of the isoelectric gateway remains substantially constant during application of the electric potential.

- 22. (Original) The method according to claim 21 wherein a pH gradient is formed between the electrodes.
- (Currently Amended) The method according to claim 21 wherein the first 23. selective barrier is a second isoelectric gateway which separates the first and second sample chambers, wherein the second isoelectric gateway is comprised of a third ionpermeable barrier having a characteristic pore size substantially restricting passage of molecules having a specificed size larger than the characteristic pore size of the barrier and a fourth ion-permeable barrier having a characteristic pore size substantially restricting passage of molecules having a specified size larger than the characteristic pore size of the barrier to form an isoelectric gateway, wherein the third ion-permeable barrier and the fourth ion-permeable barrier are selected from the group consisting of porous solids, non-ionic membranes, isoelectric membranes, non-ionic gels, and isoelectric gels, wherein the third ion-permeable barrier is positioned at a distance apart from the fourth ion-permeable barrier so as to define a space therebetween, an isoelectric substance disposed in the space between the third and fourth ion-permeable barriers forming the second isoelectric gateway, and wherein the isoelectric substance has a characteristic pI value and molecular weight such that the isoelectric substance cannot pass through the

third and fourth ion-permeable barriers a characteristic size larger than the pore size of the third and fourth ion-permeable barriers, and wherein the third and fourth ion-permeable barriers substantially restrict movement of the isoelectric substance through the third and fourth ion-permeable barriers and substantially prevent convective mixing between the contents of the second isoelectric gateway, the first sample chamber, and the second sample chamber.

(Currently Amended) The method according to claim 21 wherein the second 24. selective barrier is a second isoelectric gateway which separates the second sample chamber from the second electrolyte chamber, wherein the second isoelectric gateway is comprised of a third ion-permeable barrier having a characteristic pore size substantiallycapable of restricting passage of molecules having of a specified size larger than the characteristic pore size of the barrier and a fourth ion-permeable barrier having a characteristic pore size substantially capable of restricting passage of molecules having of a specified size larger than the characteristic pore size of the barrierto form an isoelectric gateway, wherein the third ion-permeable barrier and the fourth ion-permeable barrier are selected from the group consisting of porous solids, non-ionic membranes, isoelectric membranes, non-ionic gels, and isoelectric gels, wherein the third ion-permeable barrier is positioned at a distance apart from the fourth ion-permeable barrier so as to define a space therebetween, an isoelectric substance disposed in the space between the third and fourth ion-permeable barriers forming the second isoelectric gateway, and wherein the isoelectric substance has a characteristic pI value and molecular weight such that the isoelectric substance cannot pass through the third and fourth ion-permeable barriers a

eharacteristic size larger than the pore size of the third and fourth ion-permeable barriers, and wherein the third and fourth ion-permeable barriers substantially restrict movement of the isoelectric substance through the third and fourth ion-permeable barriers and substantially prevent convective mixing between the contents of the second isoelectric gatev/ay, the second sample chamber, and the second electrolyte chamber.

25. (Currently Amended) The method according to claim 21 wherein

the first selective barrier is a second isoelectric gateway which separates the first and second sample chambers, wherein the second isoelectric gateway is comprised of a third ion-permeable barrier having a characteristic pore size substantially capable of restricting passage of molecules having a specified size larger than the characteristic pore size of the barrier and a fourth ion-permeable barrier having a characteristic pore size substantially capable of restricting passage of molecules having of a specified size larger than the characteristic pore size of the barrier form an isoelectric gateway, wherein the third ion-permeable barrier and the fourth ion-permeable barrier are selected from the group consisting of porous solids, non-ionic membranes, isoelectric membranes, non-ionic gels, and isoelectric gels, wherein the third ion-permeable barrier is positioned at a distance apart from the fourth ion-permeable barrier so as to define a space therebetween, an isoelectric substance disposed in the space between the third and fourth ion-permeable barriers forming the second isoelectric gateway, and wherein the isoelectric substance has a characteristic pI value and molecular weight such that the isoelectric substance cannot

pass through the third and fourth ion-permeable barriers a characteristic size larger than the pore size of the third and fourth ion-permeable barriers, and wherein the third and fourth ion-permeable barriers substantially restrict movement of the isoelectric substance through the third and fourth ion-permeable barriers and substantially prevent convective mixing between the contents of the second isoelectric gateway, the first sample chamber, and the second sample chamber; and

the second selective barrier is a third isoelectric gateway which separates the second sample chamber from the second electrolyte chamber, wherein the third isoelectric gateway is comprised a fifth ion-permeable barrier having a characteristic pore size substantially capable of restricting passage of molecules having of a specified size larger than the characteristic pore size of the barrier and a sixth ion-permeable barrier having a characteristic pore size substantially capable of restricting passage of molecules havingof a specified size larger than the characteristic pore size of the barrierte form an isoel setric gateway, wherein the fifth ion-permeable barrier and the sixth ion-permeable barrier are selected from the group consisting of porous solids, non-ionic membranes, isoelectric membranes, non-ionic gels, and isoelectric gels, wherein the fifth ionpermeable barrier is positioned at a distance apart from the sixth ion-permeable barrier so as to define a space therebetween, an isoelectric substance disposed in the space between the fifth and sixth ion-permeable barriers forming the third isoelectric gateway, and wherein the isoelectric substance has a characteristic pI value and molecular weight such that the isoelectric substance cannot pass through the fifth and sixth ion-permeable barriersa characteristic size larger than the pore size of the fifth and sixth ion-permeable barriers, and wherein the fifth and sixth ion-permeable barriers substantially restrict movement of the isoelectric substance through the fifth and sixth ion-permeable barriers and substantially prevent convective mixing between the contents of the third isoelectric gateway, the second sample chamber, and the second electrolyte chamber.

56104576-51

26. (Currently Amended) The isoelectric gateway according to claim 1 wherein the isoelectric substance has a buffering capacity and conductivity around its characteristic pI value.